Title

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Journal

Advanced Materials, 30(29)

ISSN

0935-9648

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Publication Date

2018-07-01

DOI

10.1002/adma.201800927

Peer reviewed

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A 3D Magnetic Hyaluronic Acid Hydrogel for Magnetomechanical Neuromodulation of Primary Dorsal Root Ganglion Neurons

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Neuromodulation tools are useful to decipher and modulate neural circuitries implicated in functions and diseases. Existing electrical and chemical tools cannot offer specific neural modulation while optogenetics has limitations for deep tissue interfaces, which might be overcome by miniaturized optoelectronic devices in the future. Here, a 3D magnetic hyaluronic hydrogel is described that offers noninvasive neuromodulation via magnetomechanical stimulation of primary dorsal root ganglion (DRG) neurons. The hydrogel shares similar biochemical and biophysical properties as the extracellular matrix of spinal cord, facilitating healthy growth of functional neurites and expression of excitatory and inhibitory ion channels. By testing with different neurotoxins, and micropillar substrate deflections with electrophysical recordings, it is found that acute magnetomechanical stimulation induces calcium influx in DRG neurons primarily via endogenous, mechanosensitive TRPV4 and PIEZO2 channels. Next, capitalizing on the receptor adaptation characteristic of DRG neurons, chronic magnetomechanical stimulation is performed and found that it reduces the expression of PIEZO2 channels, which can be useful for modulating pain where mechanosensitive channels are typically overexpressed. A general strategy is thus offered for neuroscientists and material scientists to fabricate 3D magnetic biomaterials tailored to different types of excitable cells for remote magnetomechanical modulation.

Changes in intracellular calcium (Ca^{2+}) levels are known to affect cell signaling and downstream processes such as synaptic plasticity.[1] Consequently, there is significant interest in modulating Ca^{2+} influx in local circuits to study neural communication. Conventional tools such as electrodes and chemicals are useful for global neural stimulation but are unsuitable to study specific sub-populations of neurons.[2] Advances in optogenetics have enabled targeted neural network activation/inhibition but optical approaches are still limited by poor penetration of visible light into deep tissues.[3]

To address the limitations of existing techniques, new methods have emerged to capitalize on the sensitivity of ion channels to heat and/or mechanical forces, especially induced through magnetic field driven stimuli, to perform noninvasive neuromodulation.[4] Chen et al.[5] demonstrated the use of alternating magnetic fields to generate heat for opening TRPV1 ion channels. It remains a concern that thermogenetics may damage tissue due to sustained heating and heating from internalized magnetic nanoparticles (MNPs) at noxiously high temperatures of 43 °C.[4] Stanley et al. and Wheeler et al. recently introduced the magnetogenetics concept which utilizes magnetic forces to gate mechanosensitive ion channels.[6,7] Although the authors indicated that ferritin proteins were crystallized to exert mechanical forces on mechanosensitive ion channels, it is expected that the exerted force would be small due to the small size of the ferritin nanoparticles (5–10 nm), thus raising questions about the origin of Ca^{2+} influx with their technique.[8] All these emerging techniques also rely on exogenous gene transfections that could disrupt existing network homeostasis[9] and the choice of transfection methods might also affect their suitability for clinical/translational use (Table S1, Supporting Information).

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DOI: 10.1002/adma.201800927

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By amplifying magnetic field gradients in the local vicinity of cells using microscale ferromagnetic elements, we demonstrated previously that membrane-bound MNPs could stretch the lipid bilayer to enhance the opening probability of endogenous mechanosensitive N-type Ca^{2+} channels to induce calcium influx in cortical neural networks.[10] Nonetheless, our micromagnetic substrate with a Young's modulus on the order of GPa is mechanically incompatible for use in soft brain tissues.[11] This motivated us to develop new magnetic biomaterials that allow exploiting the same underlying mechanism of magnetomechanical neuromodulation.

Here, we describe a 3D magnetic hydrogel composed of hyaluronic acid (HA) with similar biochemical and biophysical properties to native brain/spinal cord extracellular matrix.[12] The magnetic HA gel was used for acute and chronic magnetomechanical modulation of primary rat dorsal root ganglion (DRG) neurons that expressed high density of endogenous mechanosensitive PIEZO2 and TRPV4 ion channels. Capitalizing on the receptor adaptation phenomenon characteristics of DRG neurons, we also demonstrated the utility of chronic magnetomechanical stimulation for reducing the expression of mechanosensitive PIEZO2 channels which are typically overexpressed patients suffering from chronic pain. We believe that these magnetic HA hydrogels have potential for future use in several applications including remote neural stimulation and regenerative medicine.

Fabrication of magnetic hyaluronic acid hydrogel: The magnetic HA gel is synthesized by reacting 4-arm-polyethylene glycol vinylsulfone (PEG) with high molecular weight (700 kDa) HA-thiol, a main component of the brain/spinal cord extracellular matrix,[12] and 1 µm diameter fluorescent magnetic microparticles (MMPs, Figure S2A,B, Video S1, Supporting Information) functionalized with thiol (**Figure 1**A). Through analyzing fluorescence images, we found that the

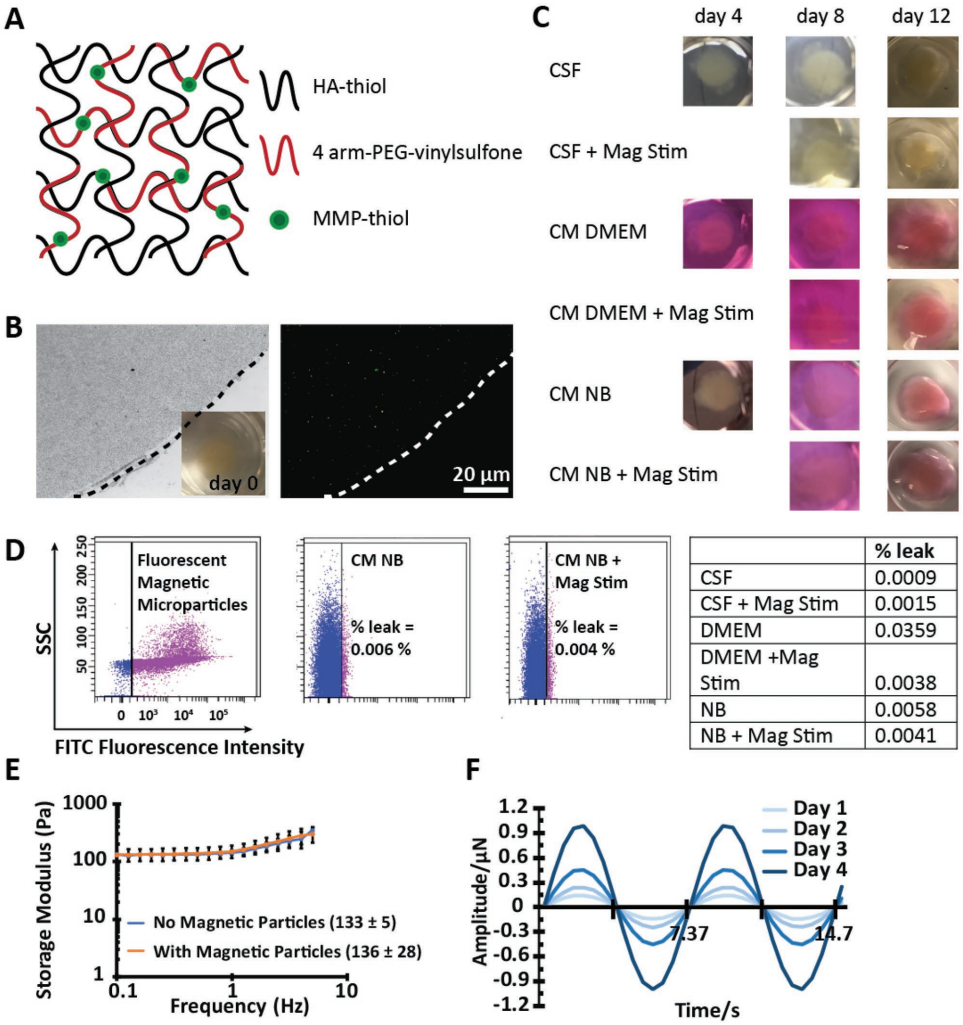

Figure 1. Characterization of magnetic HA hydrogels. A) Schematic of magnetic HA hydrogels. Magnetic microparticles conjugated with thiol were first reacted with 4-arm-PEG-VS before mixing with HA-thiol to form magnetic HA hydrogels. MMP-thiol shown is not to scale. B) Bright-field and fluorescence images showing even distribution of MMPs in HA hydrogels about 5 µm apart from one another. C) Size and shape profiles of hydrogels under different conditions. CM: conditioned media. The hydrogels were stable for at least 12 days. D) Flow cytometry analysis showed that there was minimal leakage of magnetic microparticles from the magnetic HA hydrogels in different solutions. This was important to minimize cytotoxicity and ensure consistent magnetic forces were transduced to the DRG neurons. E) The storage modulus of magnetic HA hydrogels of 136 ± 28 Pa was similar to that of the brain/spinal cord extracellular matrix (100–200 Pa). F) Forces applied to magnetic HA hydrogels over different days of magnetomechanical stimulation at 0.136 Hz

fluorescent MMPs were evenly distributed within the hydrogels with an average distance of ≈5 μm apart from one another (Figure 1B).

We assessed the stability of the hydrogels in different media commonly used in electrophysiology experiments and for culturing neurons. The hydrogels were stable, with minimal change in physical size/shape at day 12 even with 4 days of magnetomechanical perturbations with a permanent magnet (Figure 1C). Cell-internalized MMPs can cause cytotoxicity.[13] MMP leakage from the hydrogels would also reduce the magnitude of mechanical forces that could be applied. We thus quantified the percentage of fluorescent MMPs in the surrounding solution of the hydrogels using fluorescence flow cytometry and found negligible (<1%) MMP leakage from the magnetic HA hydrogels across all conditions investigated (Figure 1D).

To ensure healthy neuronal growth in the magnetic HA hydrogels, it is paramount to optimize both the biochemical and biophysical properties.[14] We hence synthesized the magnetic HA hydrogels using high molecular weight (700 kDa) HA which are also abundant in brain/spinal cord extracellular matrix.[12] Cells including neurons respond to biophysical cues which influence their growth and regeneration.[15] Therefore, we optimized the concentrations of HA-thiol and MMPs-thiol to obtain magnetic HA hydrogels with an average storage modulus of ≈136 ± 28 Pa (Figure 1E) to mimic that of the brain/spinal cord extracellular matrix of 100–200 Pa.[16]

Previously, we found that chronic magnetomechanical stimulation with increasing force magnitude/day modulated the expression of mechanosensitive N-type Ca^{2+} channels that were overexpressed in Fragile X Syndrome-model primary cortical neural networks.[10] Capitalizing on this finding, we decided to chronically stimulate the DRG neurons cultured in magnetic hydrogels for four days for 30 min per day at 0.136 Hz (Figure 1F; Figure S5, Video S3, Supporting Information) with increasing force magnitudes (0.15 to 1 μN, Equation (S1), Tables S2 and S3, Supporting Information). As the hydrogels were firmly attached to the bottom of well-plates, magnetic forces were transduced into mechanical stimulations on DRG neurons. Based on our fluorescent images that MMPs are evenly distributed ≈5 μm apart from one another (Figure 1B) and theoretical calculations, we showed that mechanical stresses were evenly distributed in the magnetic HA hydrogels, thus allowing both somas and neurites to be mechanically stimulated (Equation (S4), Figure S8, Supporting Information).

Hydrogels support viable cultures of primary neurons and extension of functional neurites: Before applying the magnetic HA hydrogels for neural stimulation, we assessed the health of primary DRG neurons cultured in 3D within the hydrogels. We observed no significant difference in cytotoxicity compared with 12-day-old neurons cultured on standard 2D poly-L-lysine-coated coverslips, 3D HA gels, and 3D magnetic HA gels with or without chronic magnetomechanical stimulation with a live/dead assay (**Figure 2**A). The metabolic activities of the neurons were also similar across different gel conditions (Figure 2B).

We found that DRG neurons displayed healthy primary neurite outgrowths in (non-)magnetic HA hydrogels (Figure 2C) with observable penetration and growth in the gel networks (Figure S6A,B, Supporting Information). On average, neurons cultured under different conditions, i.e., (non-) magnetic hydrogels (non-) stimulated had typical 4–7 primary neurites, indicating healthy development (Figure 2D).

Finally, we found that all the DRG neurons had similar absolute fluorescence levels when loaded with a Ca^{2+}-sensitive dye (Figure 2E), fluo-4 (Figure S6C, Supporting Information). This test allowed us to know that the neurons were growing healthily in the hydrogels and were not experiencing excessive stresses that typically led to heightened intracellular calcium levels. 12-day-old neurons cultured in various conditions also exhibited a similar increase in intracellular fluorescence Ca^{2+} levels after incubation with bicuculline ($EC_{50} = 3.0 \times 10^{-6}$ M), suggesting that they expressed similar and healthy levels of inhibitory γ-Aminobutyric ($GABA_A$) receptors (Figure 2F).

Acute magnetomechanical neural stimulation induced calcium influx: Primary DRG neurons express a number of endogenous mechanosensitive ion channels.[17] Four of them, i.e., PIEZO1, PIEZO2, TRPV4, and N-type Ca^{2+} have been shown to be activatable by magnetomechanical stimulation.[4]

We performed immunolabeling for the four mechanosensitive ion channels and found that consistent with the literature, primary DRG neurons express higher densities of PIEZO2 than PIEZO1 (**Figure 3**A,B).[18] We also found that the DRG neurons had high expression of TRVP4 (Figure 3C) but a low expression of N-type Ca^{2+} channels which are primarily concentrated at the axonal boutons and synapses at low (Figure 3D) and high density neural networks (Figure 3E).

We found a 50% ± 5.1% increase in $\Delta F/F_0$ (change in fluorescence Ca^{2+} levels over background fluorescence) after acute magnetomechanical stimulation (Figure 3F). Note that for subsequent experiments, drugs/inhibitors were added for the condition of stimulated magnetic HA hydrogels. To investigate whether our method of stimulation was voltage-dependent, we first added 1×10^{-6} M tetrodotoxin (TTX), a highly specific inhibitor of voltage-gated sodium channels involved in action potential propagation. We observed apparent stimulatory effects due to magnetomechanical forces even with TTX (Figure 3F), suggesting that neural stimulation was not voltage-dependent. Next, we inhibited the mechanosensitive N-type Ca^{2+} channels with a highly specific inhibitor, ω-conotoxin-GVIA.[19] There was no significant difference in $\Delta F/F_0$ in the presence of ω-conotoxin-GVIA after acute magnetomechanical stimulation (Figure 3F). This suggests that N-type Ca^{2+} channels most likely did not contribute or contributed very little to calcium influx, consistent with our previous finding that the expression density of mechanosensitive ion channels affected their contribution to Ca^{2+} influx after magnetomechanical neural stimulation.[10]

We hypothesized that if that was the case for N-type Ca^{2+} channels, because of its low expression, PIEZO1 would also have no or minimal contribution to Ca^{2+} influx after acute magnetomechanical stimulation. Unlike N-type Ca^{2+} channels, there is no specific inhibitor against PIEZO1 without also inhibiting PIEZO2 and TRPV4 (Table S3, Supporting Information). However, Yoda1 is a specific activator of PIEZO1.[20] We reasoned that if we observed significantly lower $\Delta F/F_0$ in the presence of Yoda1 which would have activated PIEZO1, it meant that PIEZO1 was a major contributor to intracellular change in Ca^{2+} level. As expected, $\Delta F/F_0$ was not significantly different from treatment with Yoda1, suggesting that PIEZO1 channels

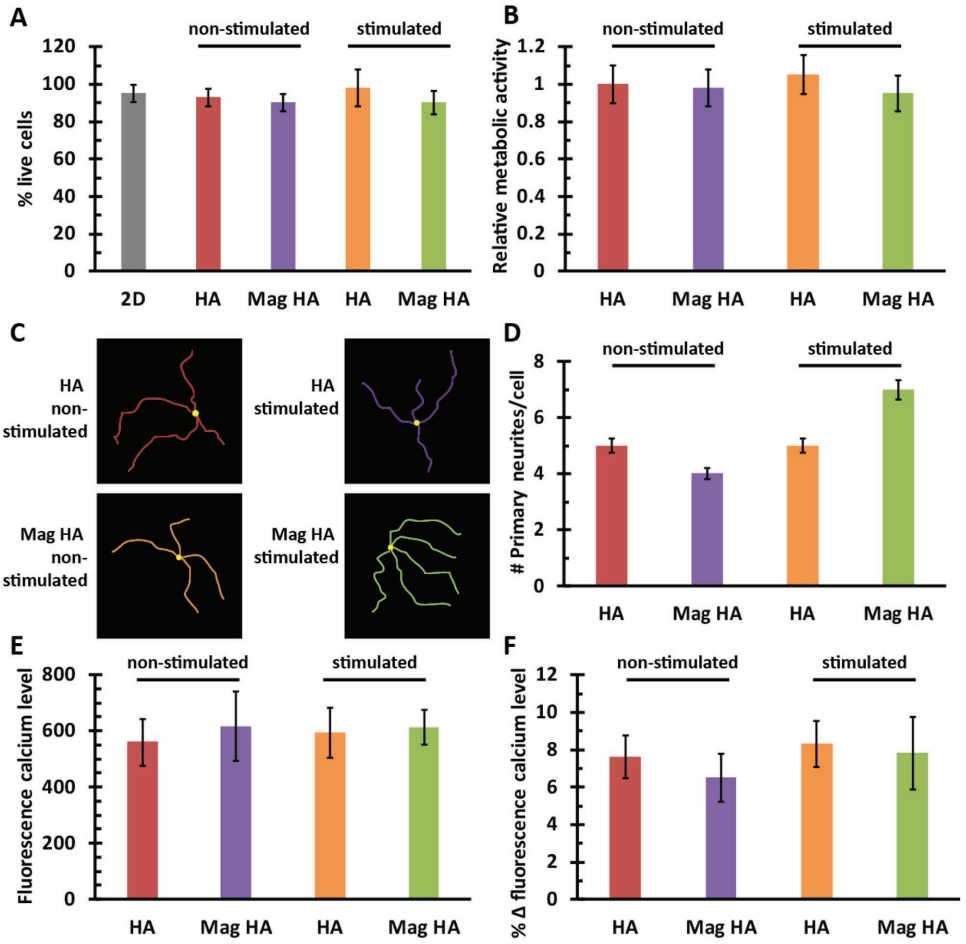

Figure 2. Healthy neuronal development in hydrogels. A) Live/dead assay showed that there was minimal cytotoxicity and high survival rate of DRG neurons in the hydrogels. B) Metabolic activities of DRG neurons were similar in different hydrogels. C) Neurite Tracer images showing primary neurites of DRG neurons cultured in different hydrogels. D) Average number of primary neurites of DRG neurons cultured in different hydrogels was between 4 and 7, indicating healthy neuronal growth. E) Similar fluorescence Ca^{2+} levels in resting state and F) similar increase in fluorescence Ca^{2+} levels after treatments with bicuculline suggested functional neuronal developments in different hydrogels. HA: hyaluronic acid hydrogels; Mag HA: magnetic hyaluronic acid hydrogels. Number of cells represented in (A), (B), (E), and (F): 50 000 cells per condition which corresponds to the total number of cells per well. Triplicates were performed for each condition. Number of cells represented in (D): 30 cells per condition.

had minimal contribution to Ca^{2+} influx during magnetomechanical neural stimulation (Figure 3F). This is supported by a recent report that PIEZO1 channels act as high-pass filters and are activated only at mechanical stimulation frequency ≥10 Hz[21] while our stimulation was <1 Hz. We next inhibited both PIEZO2 and TRPV4 with Ruthenium red and found that this significantly negated the effects of mechanical neural stimulation (Figure 3F).

There is no specific inhibitor against PIEZO2. GSK205, which has been reported to inhibit TRPV4,[22] is not widely verified and has not been tested for its inhibition against other channels in the TRP family. To overcome the lack of specific neuroinhibitors, we sought to determine whether forces applied through magnetic/mechanical stimulation and transduced via hydrogel deformations could gate these mechanosensitive channels.

With our magnetic HA hydrogels, we could magnetically actuate the MMPs which then presumably stretched cell membranes to activate PIEZO2.[21,23] Servin-Vences et al.[22] found that while PIEZO could be activated by small membrane stretch (90 mmHg), TRPV4 did not. However, TRPV4 was highly sensitive to deflection at the cell–substrate interface.[22] HA is a bioactive polymer that binds to cell surface receptors, and magnetic actuation is expected to activate TRPV4 through force transduction through these receptors.[12] To test our hypothesis, we performed substrate deflection using a micropillar array[24] (**Figure 4**A,B) on HEK cells with tetracycline-inducible TRPV4 expression.[25]

As expected, when we applied larger deflections, currents of higher amplitudes were recorded[22,24] (Figure 4C,D). Current is a function of N (number of ion channels), g (conductance of channels), and P_0 (opening probability). However, N is not expected to change significantly during mechanical stimulation that lasts on the order of milliseconds and g which is a measure of evolutionarily conserved pore structure is also unlikely to change significantly.[10] Therefore, it is most likely that larger forces increased P_0, similar to findings from published literature,[22,24] leading to larger currents.

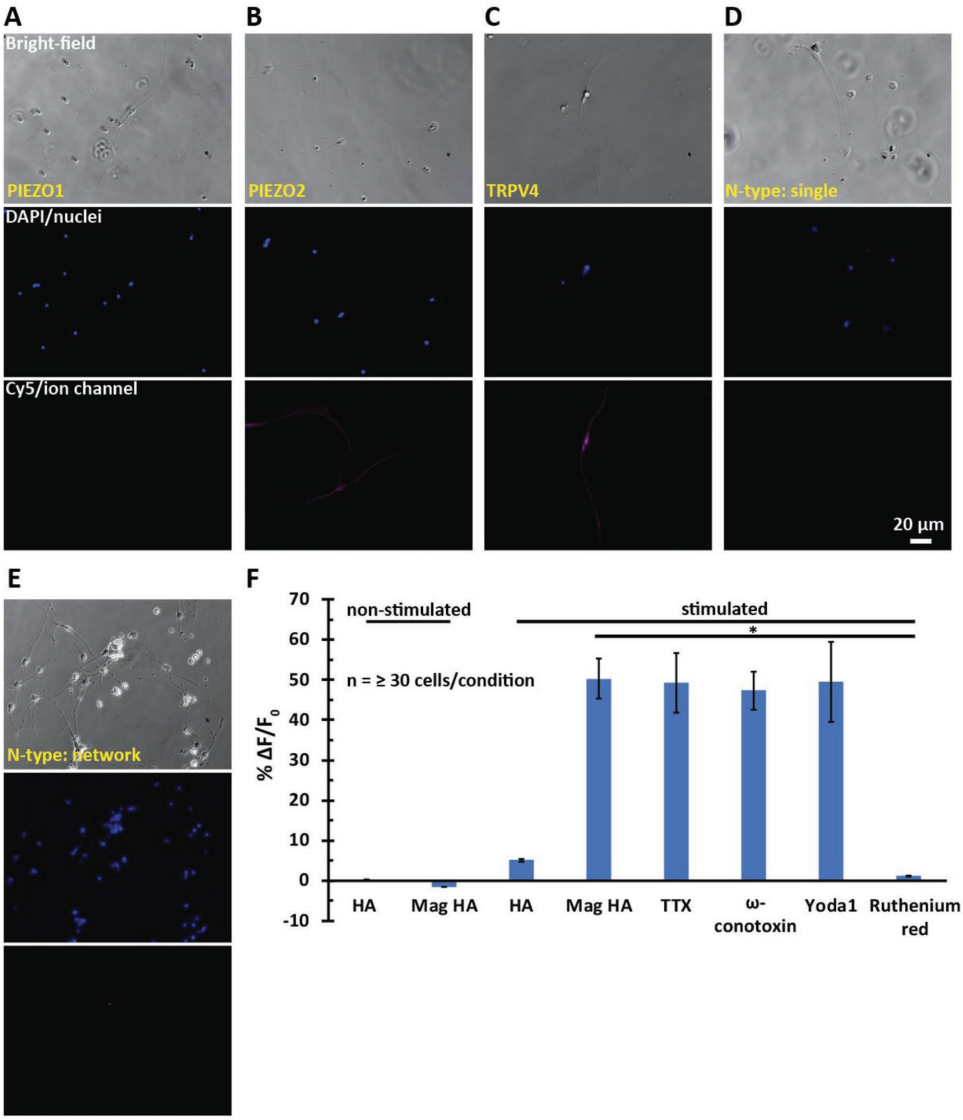

Figure 3. Acute and chronic magnetomechanical stimulation. A,B) There was low expression of PIEZO1 channels (A) compared to much higher expression of PIEZO2 channels (B) on DRG neurons. C–E) High expression of TRPV4 channels (C), but low expression of N-type Ca^{2+} channels in single neurons (D) or neural networks (E). F) Magnetomechanical stimulation induced Ca^{2+} influx in magnetic HA hydrogels with $\Delta F/F_0 = 50\% \pm 5.1\%$. Stimulation was independent of ω-conotoxin (N-type Ca^{2+} channels), Yoda1 (PIEZO1 channels). Ca^{2+} influx was inhibited by Ruthenium red that blocks both PIEZO2 and TRPV4 channels. Ca^{2+} fluorescence signals with Ruthenium red was statistically significantly different from those with no toxins added, TTX, ω-conotoxin, and Yoda1. HA: hyaluronic acid hydrogels; Mag HA: magnetic hyaluronic acid hydrogels; Nstim: not stimulated; Stim: stimulated. *: $p < 0.05$.

Based on the results of: (1) pharmacological inhibitions, (2) electrophysiological data, (3) our previous findings and existing literature that Ca^{2+} influx is attributed mostly to highly expressed endogenous mechanosensitive channels and (4) that PIEZO2 channels are activated by forces at low frequency,[21] we reasoned that PIEZO2 and TRPV4 which were highly expressed on primary DRG neurons likely contributed the most to Ca^{2+} influx after magnetomechanical neural stimulation.

Chronic magnetomechanical stimulation modulated expression of mechanosensitive PIEZO2 channels: It is well-known that neural networks actively regulate their ratio of excitatory to inhibitory ion channels/receptors to maintain network homeostasis.[26] Previously, we found that Fragile X Syndrome-model primary cortical neural networks reduced their expressions of mechanosensitive N-type Ca^{2+} channels after chronic magnetomechanical stimulation only with increasing force magnitudes.[10] We wanted to examine whether this phenomenon could also be observed in primary DRG neural network.

We performed chronic mechanical stimulation for 4 days for 30 min per day (day 1: 0.145 µN, day 2: 0.244 µN, day 3: 0.457 µN, day 4: 1.00 µN, Figure 1F). Our decision to increase the force magnitude daily is also supported by the phenomenon of receptor adaptation where DRG neurons respond to stable stimuli with decreasing frequencies of action potentials and stronger stimuli are needed for continual excitation for neuromodulation.[27]

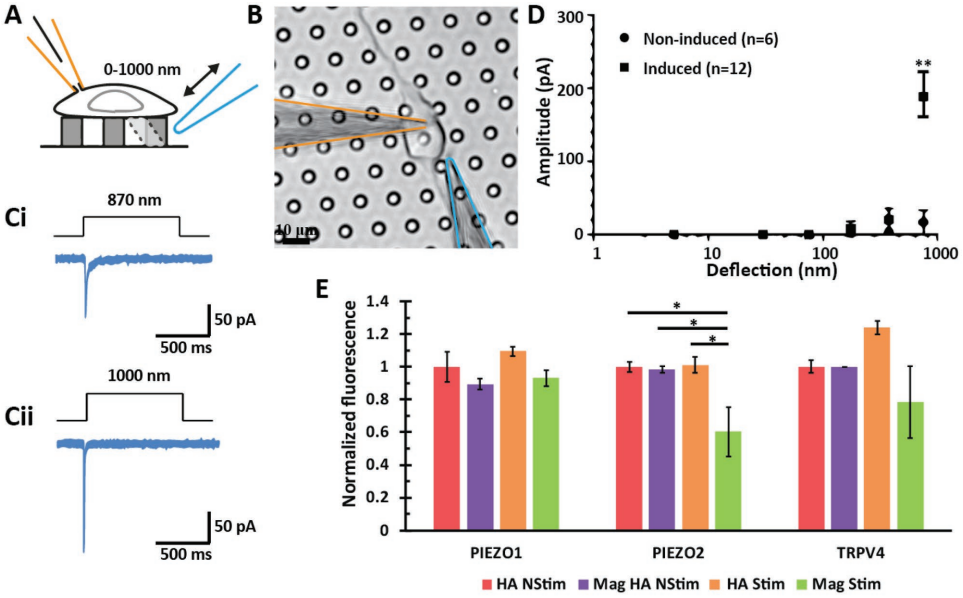

Figure 4. Electrophysiological recordings and chronic magnetic stimulations. A) Schematic of micropillar deflection with concurrent electrophysiological recordings. B) Bright-field image of HEK cells with inducible TRPV4 mechanically stimulated. C) Example traces showing that larger mechanical substrate deflections enhanced opening probability of mechanosensitive TRPV4, leading to larger current amplitudes. D) Stimulus response graph of deflection-gated currents in HEK with and without TRPV4. Measurements from an individual cell were binned according to stimulus size and current amplitudes were averaged within each bin, then across cell and data are displayed as mean ± standard mean error. HEK cells with inducible TRPV4 expression showed statistically significant robust response to mechanical substrate deflections compared to HEK cells without TRPV4 at 500–1000 nm substrate deflections. E) Chronic magnetomechanical stimulation significantly reduced the expression of PIEZO2 channels but not PIEZO1/TRPV4 channels in DRG neurons cultured in magnetic HA hydrogels. 50 000 cells were present in each well/condition. Triplicates were performed for each condition. HA: hyaluronic acid hydrogels; Mag HA: magnetic hyaluronic acid hydrogels; Nstim: not stimulated; Stim: stimulated. *: $p < 0.05$, **: $p < 0.001$.

We found that DRG neural networks cultured in magnetic HA hydrogels that underwent chronic magnetomechanical stimulation with increasing force magnitude/day over 4 days (day 9–12) had statistically significant reduction in their expression of excitatory, mechanosensitive PIEZO2 channels (Figure 4E). There was, however, no statistically significant difference in the expression of PIEZO1 and TRPV4 (Figure 4E).

Neural stimulatory techniques allow neuroscientists to investigate neurocommunication and to modulate neural circuits implicated in behaviors such as learning and feeding.[6] However, current tools for neural modulation face limitations such as non-specificity and invasiveness. Emerging methods such as thermogenetics can damage tissues especially during chronic use and the mechanism of ferritin-based magnetogenetics is still being questioned.[4]

Here, we describe a novel magnetic HA hydrogel that overcomes some of the limitations and offers advantages over existing tools: (1) The 3D magnetic HA hydrogels made use of magnetic fields with deep tissue penetration. This allows in vitro and in vivo remote, noninvasive neural stimulation across deep brain tissues and potentially neurons in the peripheral nervous system.[28] (2) The magnetic HA hydrogels share similar biochemical (use of high molecular weight 700 kDa HA) and biophysical properties (storage modulus of 136 Pa) to the native extracellular matrix of the brain/spinal cord that allow neurons to grow healthily. (3) Compared to other neural stimulatory materials such as optoelectronic devices, magnetic HA hydrogels can be injected easily into deep tissues, synthesized in large quantities and conveniently lyophilized, transported, and rehydrated for use in different laboratories. (4) The magnetic HA hydrogel has low MMP leakage which is an advantage over emerging neuromodulatory tools using freely moving magnetic particles which have reduced stimulatory efficacy and can cause cytotoxicity when their protective coating such as PEG[5] or starch are degraded with time.[29] (5) Magnetomechanical stimulation enhanced the opening probability of endogenous mechanosensitive channels that are highly expressed in neurons allowing Ca^{2+} influx. This avoids the step for exogenous ion channel transfection which can disrupt existing network homeostasis,[9] and have variable efficiency based on the age of the subjects[13] and transfection methods.[30] (6) This method can be used to magnetomechanically stimulate other neuronal-types and excitable cells such as cardiac cells that also express mechanosensitive ion channels by conjugating biomaterials with similar biochemical/biophysical properties with MMPs for tissue-specific tailored stimulation. The time for complete gel degradation can also be modified with chemical means.

We demonstrated that we could elicit Ca^{2+} influx in primary DRG neurons cultured in 3D magnetic HA hydrogels with 50% ± 5.1% increase in $\Delta F/F_0$. The mechanism of neural stimulation did not involve voltage-sensitive sodium ion channels. By inhibiting different mechanosensitive channels and understanding the force sensitivities of mechanosensitive channels, we also reasoned that PIEZO2 and TRPV4 channels which are abundant on primary DRG neurons contributed most significantly to the Ca^{2+} influx (**Figure 5**). Chronic magnetomechanical stimulation with increasing forces reduced the expression of PIEZO2 channels consistent with the receptor adaptation

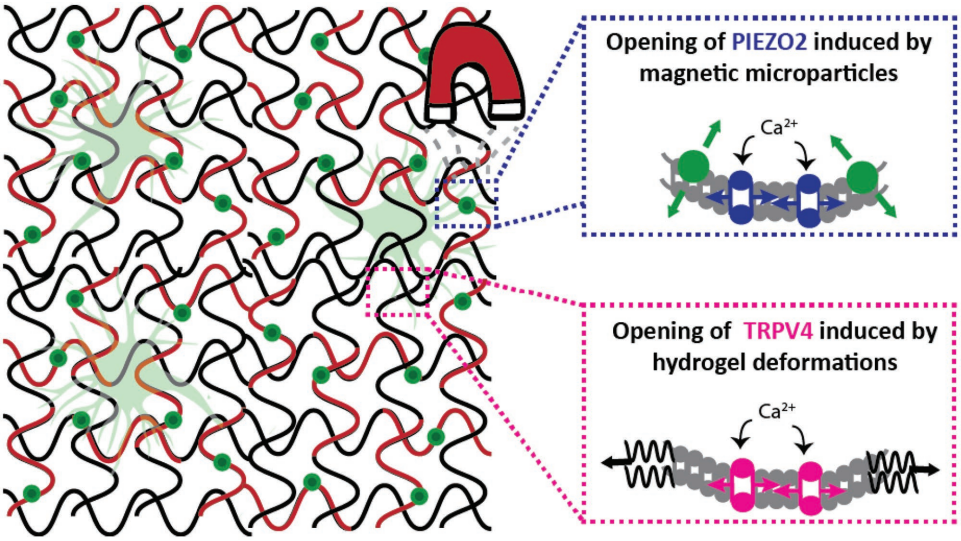

Figure 5. Mechanism of magnetomechanical stimulation of DRG neurons with magnetic hyaluronic acid hydrogels. Mechanosensitive PIEZO2 channels are activated by magnetic microparticles embedded in the hydrogels through membrane stretching. On the other hand, mechanosensitive TRPV4 channels are activated by magnetic force-induced hyaluronic acid hydrogel deformations.

phenomenon and previous finding that neural networks actively regulate their ratio of excitatory to inhibitory signals to maintain homeostasis.[10]

We believe that other than being a tool for acute neural stimulation, the magnetic HA hydrogels might also find utility in chronic pain modulation, by reducing the expression of mechanosensitive PIEZO2 channels.[31] The magnetic HA hydrogels could also be used to study the role of mechanotransduction in tissue regeneration.[32] In the future, it will be important to investigate the effects of different force magnitudes and how there might be force-sensitivity differences amongst the various mechanosensitive channels.

Experimental Section

Hydrogel Fabrication and Cell Encapsulation: Thiol-functionalized magnetic microparticles (MMP-SH) were conjugated to 4-arm-polyethylene-glycol-vinyl-sulfone (PEG-VS, 20 kDa, JenKem Technologies) prior to hydrogel fabrication. Briefly, MMP-SH (500×10^{-3} M) were added to a solution of PEG-VS, 20 mg mL^{-1}, 20×10^{-3} M HEPES, pH = 7) and kept at 37 °C for 2 h to achieve PEG-MMP. HA-SH (M_w = 700 kDa) was dissolved at 20 mg mL^{-1} (20×10^{-3} M, HEPES, pH = 10) where the pH was later adjusted to 7 using 1 M NaOH. Equal volumes of HA-SH and PEG-MMP solutions were mixed and 40 µL of the mixed solution was injected to a 96 well-plate followed by 90 min of incubation at 37 °C. To encapsulate the neurons, aliquots of neurons (4 µL, 50 000 cells) were injected into each hydrogel 45 min into gelation. Cell-laden hydrogels were incubated for another 45 min at 37 °C until full gelation is achieved before addition of culture media (150 µL per well in a 96 well-plate).

Dissociation of Neurons from Primary Dorsal Root Ganglions Neuron: Embryonic primary dorsal root ganglions (DRGs) were purchased from Brainbits. Embryonic primary DRGs tissues were placed in cell dissociation solution (1 mg mL^{-1} collagenase/8 mg mL^{-1} dispase) at 37 °C for 1 h with gentle swirling every 5 min. After removal of cell dissociation solution, 2 mL of Hibernate AB (Brainbits) were added. Tissue was triturated ≈30 times using a 1 mL pipette tip and centrifuged at 200 g for 3 min. The supernatant was removed and appropriate volume of culture media was used to resuspend the pellet. Culture cells with Neurobasal media (Life Technologies) supplemented with 10% B27, 1% GlutaMAX and 25 ng mL^{-1} nerve growth factor (Brainbits). Neurons were cultured in 37 °C incubator maintained at 5% CO_2 with addition of 50% media change every 7 days.

Calcium Dye Incubation and Magnetic Force Stimulation: Primary DRG neurons were incubated with Fluo-4 Direct calcium assay kit with 250×10^{-3} M stock solution of probenecid. Briefly, 5 mL of calcium assay buffer was mixed and vortexed with 100 µL of probenecid stock solution to create a 2× loading dye solution. The dye solution was then added to the cells with media in a 1:1 ratio and incubated for 1 h before imaging to allow sufficient diffusion through the hydrogels. For experiments involving TTX (1×10^{-6} M), ω-conotoxin GVIA (1×10^{-6} M), bicuculline (3×10^{-6} M), Yoda1 (25×10^{-6} M), and Ruthenium red (30×10^{-6} M), the chemical was added during calcium dye incubation (1 h).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was performed with funding from the US National Institutes of Health Director's New Innovator Award (1DP2OD007113). A.T. received the Endeavour Research Fellowship and IBRO-APRC Fellowship to perform electrophysiological recordings at UNSW. The authors acknowledge the use of instruments at the Electron Imaging Center for NanoMachines supported by NIH (1S10RR23057 to ZHZ). HEK cells with inducible TRPV4 expressions with tetracycline were a kind gift from Shireen Lamandé and Peter McIntyre. A.T. fabricated magnetic HA hydrogel, characterization, acute and chronic magnetic stimulations, and electrophysiological recordings and performed experiments and analyzed data. A.S. fabricated magnetic HA hydrogel and performed rheological measurements. A.T. wrote the manuscript and all authors revised the manuscript. Embryonic primary dorsal root ganglions (DRGs) from E18 rat embryos used in this work were purchased from Brainbits.

Note: The first name of author Alireza Sohrabi was corrected in the author byline on July 16, 2018, after initial publication online.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomaterials, hyaluronic acid, hydrogels, magnetic materials, neural modulation

Received: February 8, 2018
Revised: April 20, 2018
Published online: June 10, 2018

[1] M. J. Berridge, M. D. Bootman, P. Lipp, *Nature* **1998**, *395*, 645.
[2] M. Banghart, K. Borges, E. Isacoff, D. Trauner, R. H. Kramer, *Nat. Neurosci.* **2004**, *7*, 1381.
[3] D. R. Sparta, A. M. Stamatakis, J. L. Phillips, N. Hovelsø, R. van Zessen, G. D. Stuber, *Nat. Protoc.* **2012**, *7*, 12.
[4] D. Di Carlo, A. K. Tay, *Curr. Med. Chem.* **2016**, *24*, 537.
[5] R. Chen, G. Romero, M. G. Christiansen, A. Mohr, P. Anikeeva, *Science* **2015**, *347*, 1477.
[6] S. A. Stanley, L. Kelly, K. N. Latcha, S. F. Schmidt, X. Yu, A. R. Nectow, J. Sauer, J. P. Dyke, J. S. Dordick, J. M. Friedman, *Nature* **2016**, *531*, 647.
[7] M. A. Wheeler, C. J. Smith, M. Ottolini, B. S. Barker, A. M. Purohit, R. M. Grippo, R. P. Gaykema, A. J. Spano, M. P. Beenhakker, S. Kucenas, M. K. Patel, C. D. Deppmann, A. D. Güler, *Nat. Neurosci.* **2016**, *19*, 756.
[8] M. Meister, *eLife* **2016**, *5*, e17210.
[9] T. M. Otchy, S. B. E. Wolff, J. Y. Rhee, C. Pehlevan, R. Kawai, A. Kempf, S. M. H. Gobes, B. P. Ölveczky, *Nature* **2015**, *528*, 358.
[10] A. Tay, D. Di Carlo, *Nano Lett.* **2017**, *17*, 886.
[11] I. Levental, P. C. Georges, P. A. Janmey, P. A. Janmey, V. M. Laurent, B. Louis, D. Isabey, E. Planus, B. Senger, C. Picart, *Soft Matter* **2007**, *3*, 299.
[12] A. Bignami, M. Hosley, D. Dahl, *Anat. Embryol.* **1993**, *188*, 419.
[13] A. Tay, A. Kunze, D. Jun, E. Hoek, D. Di Carlo, *Small* **2016**, *12*, 3559.
[14] A. Balgude, *Biomaterials* **2001**, *22*, 1077.
[15] C. E. Schmidt, J. B. Leach, *Annu. Rev. Biomed. Eng.* **2003**, *5*, 293.
[16] P. C. Georges, W. J. Miller, D. F. Meaney, E. S. Sawyer, P. A. Janmey, J. J. Pastore, H. Falet, K. Hoffmeister, R. Kuuse, R. Uibo, J. Herod, E. Sawyer, P. A. Janmey, *Biophys. J.* **2006**, *90*, 3012.
[17] G. C. McCarter, D. B. Reichling, J. D. Levine, *Neurosci. Lett.* **1999**, *273*, 179.
[18] S. S. Ranade, R. Syeda, A. Patapoutian, *Neuroendocrinology* **2015**, *87*, 1162.
[19] E. W. McCleskey, A. P. Fox, D. H. Feldman, L. J. Cruz, B. M. Olivera, R. W. Tsien, D. Yoshikami, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 4327.
[20] R. Syeda, J. Xu, A. E. Dubin, B. Coste, J. Mathur, T. Huynh, J. Matzen, J. Lao, D. C. Tully, I. H. Engels, H. M. Petrassi, A. M. Schumacher, M. Montal, M. Bandell, A. Patapoutian, *eLife* **2015**, *4*, e07369.
[21] A. H. Lewis, A. F. Cui, M. F. McDonald, J. Grandl, *Cell Rep.* **2017**, *19*, 2572.
[22] M. R. Servin-Vences, M. Moroni, G. R. Lewin, K. Poole, *eLife* **2017**, *6*, https://doi.org/10.7554/eLife.21074.
[23] L. Volkers, Y. Mechioukhi, B. Coste, *Pflugers Arch. Eur. J. Physiol.* **2014**, *467*, 95.
[24] K. Poole, R. Herget, L. Lapatsina, H.-D. D. Ngo, G. R. Lewin, R. Herget, L. Lapatsina, H.-D. D. Ngo, G. R. Lewin, R. Herget, L. Lapatsina, H.-D. D. Ngo, G. R. Lewin, *Nat. Commun.* **2014**, *5*, 3520.
[25] S. R. Lamandé, Y. Yuan, I. L. Gresshoff, L. Rowley, D. Belluoccio, K. Kaluarachchi, C. B. Little, E. Botzenhart, K. Zerres, D. J. Amor, W. G. Cole, R. Savarirayan, P. McIntyre, J. F. Bateman, *Nat. Genet.* **2011**, *43*, 1142.
[26] L. N. Borodinsky, C. M. Root, J. A. Cronin, S. B. Sann, X. Gu, N. C. Spitzer, *Nature* **2004**, *429*, 523.
[27] P. Delmas, J. Hao, L. Rodat-Despoix, *Nat. Rev. Neurosci.* **2011**, *12*, 139.
[28] M. Weber, A. A. Eisen, *Muscle Nerve* **2002**, *25*, 160.
[29] A. Tay, F. E. Schweizer, D. Di Carlo, *Lab Chip* **2016**, *16*, 1962.
[30] A. Tay, N. Melosh, *PostDoc J.* **2018**, *6*, 26.
[31] R.-R. Ji, Z.-Z. Xu, Y.-J. Gao, *Nat. Rev. Drug Discovery* **2014**, *13*, 533.
[32] C. A. Cezar, E. T. Roche, H. H. Vandenburgh, G. N. Duda, C. J. Walsh, D. J. Mooney, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 1534.

